

Oviposition-repelling activity of synthetic polyacetylenes and electroantennogram responses in *Ostrinia furnacalis* (Lepidoptera: Pyralidae)

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Abstract: Oviposition-repelling activity of 11 synthetic polyacetylene compounds and electroantennograms (EAGs) responses in the Asian corn borer, *Ostrinia furnacalis* were assayed with the method of accepting egg on paper and EAG recording techniques. The results of bioassay indicated that the majority of tested compounds, except compound 1 and 10, could inhibit the oviposition behavior of *O. furnacalis*, and among these compounds compound 9 (1-phenyl-2,4-hexadiyne) was the most effective one. The inhibiting rate of compound 9 was from 61.27% to 85.71% at 20 $\mu\text{g}/\text{cm}^2$ in 6 days of treatment. EAGs of the moths tested indicated that there were no obvious sexual differences to some tested compounds under concentration of 10 mg/mL, which could stimulate the receptors of antenna of the insect to produce action potential. Among these compounds, the most stimulating compounds were compound 6 (1-phenyl-4-(3-nitro)-phenyl-1,3-butadiyne) and compound 9: both compounds were significantly stronger in average EAG amplitude than other tested compounds, and the males responded more sensitively than females to the two compounds. The relative EAGs (S_r) of female and male to compound 6 were 68.22% and 106.60%, and to compound 9 were 199.19% and 220.60% at 10 mg/mL respectively. The dosage-responses of compounds 6 and 9 on EAGs showed that they were dose-dependent. The regression analysis of the data of two series showed that the value of depolarization amplitude of antenna receptors induced by the tested compounds correlated in some degree with inhibiting activity in behavioral test. Finally, the action target and relationship between structure and activity of polyacetylenes to *O. furnacalis* are discussed.

Key words: Polyacetylenes; *Ostrinia furnacalis*; oviposition-repelling activity; electroantennogram responses

1 INTRODUCTION

Polyacetylene compounds with conjugated triple bond system occur naturally as secondary metabolites in higher plants. More than 700 polyacetylenes had been isolated and the bioactivity of these compounds to many species of insects had been tested (Wat *et al.*, 1981; McLachlan *et al.*, 1982). It was reported that these compounds of phenylheptatriyne (PHT), *cis*-dehydromatricaria ester and tridec-1-ene-3, 5, 7, 11-pentane, which were isolated from the plants of Asteraceae, have ovicide activity to the fruit fly (*Drosophila melanogaster*) and housefly (*Musca domestica*) as well as larvicide activity to a lot of species of mosquitoes and blackfly (*Simulium vittatum*), and the activities to these insects are enhanced greatly by irradiation of near UV or sunlight. Besides these activities, PHT also had the activities of

detering feeding and inhibiting growth to *Euxoa messoria* and *Manduca sexta* when this compound was incorporated into an artificial diet (Arnason *et al.*, 1986, 1987; McLachlan *et al.*, 1982). It has been found that 1-phenyl-2,4-hexadiyne (Table 1: compound 9), isolated from *Artemisia scoparia*, displays the activities of contact toxicity, repellent and inhibiting reproduction to several store-product pests and photo-toxicity to *Spodoptera litura* (Xu *et al.*, 1994); 1-phenyl-4-(3,4-methylenedioxy)-phenyl-1,3-butadiyne (Table 1: compound 5), a synthetic polyacetylene, was a powerful photodynamic larvicide to mosquito larva of *Culex quinquefasciatus* (Wan *et al.*, 2000); and 6, 6-dimethyl-2, 4-heptadiyn-1-ol (Table 1: compound 2), 2, 4-hexadiyn-1, 6-diol (Table 1: compound 3) and 3-bromo-2-propyne-1-ol (Table 1: compound 11) had strong antifeeding activity (Wan *et al.*, 2001). Electrophysiological study in our laboratory also demonstrated that those compounds could

stimulate the deterrent cell in the medial maxillary styloconic sensilla and inhibit the sugar-sensitive cell in the lateral maxillary styloconic sensilla on *Ostrinia furnacalis* (Wan *et al.*, 2001).

In order to understand further the activity of polyacetylenes to insects, we designed the experiment using *O. furnacalis* as a model insect to assay the interfering effect of polyacetylenes on oviposition behavior of *O. furnacalis*, and combined the EAG recording techniques for explaining the action target of new synthetic polyacetylene compounds related to behavioral reaction of the moth.

2 MATERIALS AND METHODS

2.1 Insect

A laboratory strain of Asian corn borer (*O. furnacalis*) was reared with artificial diet following the method described by Zhou *et al.* (1980). The adults were used in the experiments.

2.2 Chemicals

Tested 11 polyacetylene compounds (Table 1) were synthesized by LIU Zhun at Institute of Elemento-

Organic Chemistry, Nankai University, Tianjin, China, all in chemical purity $\geq 95\%$. *cis*-3-hexen-1-ol as a standard chemical of olfactory stimuli was provided by Prof. YAN Fu-Shun, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

2.3 Bioassay of oviposition-repelling activity

For determining of the oviposition-repelling activity of chemicals to *O. furnacalis*, 40 pupae (half female and half male) were putted into a cage-net (30 cm \times 20 cm \times 20 cm) for the purpose of emergence and oviposition. All tested chemicals were dissolved in acetone, which was not more than 1%. An appropriate volume of tested solution is applied to a small piece of wax paper of 12 cm². Tested solution (T) was distributed evenly on the paper, with acetone solution on the paper as control (C), and each treatment with three replications. After acetone evaporated in a few minutes, all papers including C and T were linked on a piece of big paper which was putted inside the cage. After emergence of the pupae, the eggs laid accumulatively on C- and T-paper were counted and the repelling index was calculated as $[(C - T)/C] \times 100$.

Table 1 Serial number, structural formula, name and melting point of tested compounds

Serial No.	Structural formula	Name	Melting point (°C)
1	$(\text{CH}_3)_3\text{C}-\text{C}=\text{C}-\text{C}=\text{C}-\text{C}(\text{CH}_3)_3$	2, 2, 7, 7-tetramethyl-3, 5-octadiyne	129 – 130
2	$(\text{CH}_3)_3\text{C}-\text{C}=\text{C}-\text{C}=\text{C}-\text{CH}_2\text{OH}$	6, 6-dimethyl-2, 4-heptadiyn-1-ol	Liquid
3	$\text{HOCH}_2-\text{C}=\text{C}-\text{C}=\text{C}-\text{CH}_2\text{OH}$	2, 4-hexadiyn-1, 6-diol	112
4		1-phenyl-4-(4-methoxyphenyl)-1, 3-butadiyne	94 – 95
5		1-phenyl-4-(3, 4-methylene dioxy)-phenyl-1, 3-butadiyne	105 – 106
6		1-phenyl-4-(3-nitro)-phenyl-1, 3-butadiyne	147 – 148
7		1-phenyl-4-(2-nitro)-phenyl-1, 3-butadiyne	152 – 153
8		1-phenyl-5-benzoyloxy-1, 3-pentadiyne	Liquid
9		1-phenyl-2, 4-hexadiyne	Liquid
10		<i>O</i> , <i>O</i> -diethyl- <i>O</i> -propargyl thiophosphate	Liquid
11	$\text{Br}-\text{C}\equiv\text{C}-\text{CH}_2\text{OH}$	3-bromo-2-propyne-1-ol	Liquid

2.4 EAG recording

EAG technique described by Hou and Yan (1995) was followed in this study. Tested compounds dissolved in acetone which was not more than 1% were diluted by paraffin oil to concentration of determination as stock solution. Ag-AgCl electrode was encased in glass capillary filled with Kassiling solution (Yan *et al.*, 1994). Heads of unmated male and female *O. furnacalis* were excised 2 d after emergence. The free terminal of top-cut antenna was inserted into the recording electrode and the indifferent electrode was inserted into the mandible. EAG signals were amplified 500 folds by a DC preamplifier (UN 06). Signals were viewed on an oscilloscope (HMEG, HM-203-6) and recorded on graph paper by an ink pressure recorder (Gould Recorder 220) for subsequent analysis and storage.

25 μL of each stock solution was placed on a filter paper (5 cm×0.5 cm), which was attached to a gas stimulating system. A purified air stream at the constant speed of 80 mL/min carried the odor molecules evaporating from the filter paper over the tested antenna. Stimulus duration time was 0.2 s and at least 30 s was allowed between two continuous stimuli for recovery of the EAG. EAGs from six to eight adults of each sex to all tested chemicals under load concentration of 1 mg/mL were recorded. Considering the waning of the antenna response with the time prolongation, *cis*-3-hexen-1-ol was used as a standard and tested following every two experimental stimuli. Responses to paraffin oil and acetone were also conducted.

EAG responses to all tested chemicals were evaluated by measuring the maximum amplitude of depolarization triggered by a stimulus (Hou and Yan, 1995). According to the values of amplitude, both absolute and relative EAG values were calculated and analyzed. Responses to the reference *cis*-3-hexen-1-ol was called R, the relative EAGs to a test stimuli (*Sr*) were expressed by the absolute amplitude (*Sc*) of the stimuli divided by the mean response to the references before (*R'*) and after (*R''*). The formula as follows: $Sr = 2Sc / (R' + R'')$.

3 RESULTS

3.1 Oviposition-repelling effect

The tested compounds, except compound 1 and 10, showed the oviposition-repelling activity to *O. furnacalis* treated with 20 μg/cm² (Table 2). During the first period (3 d), the repelling effect of compounds 8 and 9 were higher than that of other tested compounds. The compounds 6, 7, 8, and 9 still sustained the high repelling activity in 6 d of treatment. It was noted that compound 9 had significantly higher

repelling activity than others, though there was a phenomenon of decrease in activity with elongating of time, and the rate of oviposition-repelling had been over 60% during treatment time (Table 2).

3.2 EAG responses

The results of EAG investigation showed that the tested polyacetylene compounds stimulated the receptors of antenna of *O. furnacalis* to produce action potential (Fig. 1). In the tested compounds, the most effective stimulus compound was compounds 6 and 9 (Table 1, Fig. 1), they were significantly stronger in average EAG-amplitude (Fig. 1) than other tested compounds, and the male was more sensitive than the female in the response to the two compounds. The *Sr* of the female and the male to compound 6 was 68.22% and 106.60%, and to compound 9 was 199.19% and 220.60% respectively at 10 mg/mL (Fig. 1).

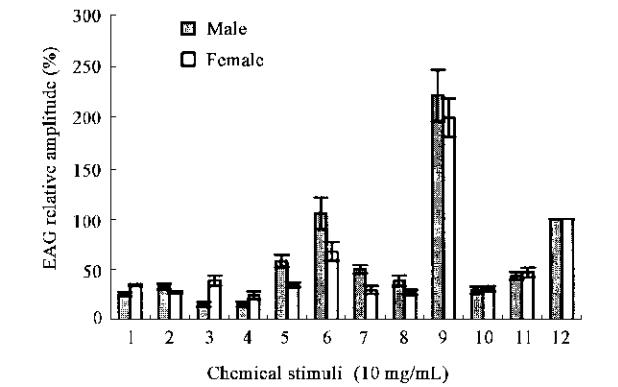


Fig. 1 EAG responses of *Ostrinia furnacalis* to tested polyacetylenes (*n* = 6 – 8)
1 – 11. See Table 1 for name of tested compounds;
12. *cis*-3-hexen-1-ol.

Table 2 The oviposition-repelling effect of polyacetylene compounds to *Ostrinia furnacalis*

No. of tested compound	Rate of oviposition-repelling (%) ($\bar{x} \pm SE$)			
	3 d	4 d	5 d	6 d
1	19.00±2.99 de	9.33±0.48 c	0.00	-40.58±0.00 b
2	33.28±0.58 cd	36.70±1.73 bc	-13.67±0.00 d	28.20±2.18 ab
3	71.42±14.01 ab	53.30±1.73 ab	38.72±2.23 ab	40.67±2.18 ab
4	47.57±12.67 bcd	46.70±1.33 abc	45.11±3.78 ab	31.30±2.41 ab
5	52.42±1.71 bc	66.70±1.33 abc	35.42±2.05 ab	34.39±7.91 ab
6	71.14±1.73 ab	80.00±1.00 a	54.79±2.71 b	59.42±8.26 a
7	66.71±10.00 ab	60.00±1.00 ab	54.79±2.71 b	56.23±5.20 a
8	81.00±4.09 a	66.70±1.44 ab	61.27±1.94 a	56.23±8.26 a
9	85.71±0.00 a	80.00±1.00 a	61.27±1.94 a	62.51±1.61 a
10	-14.28±0.00 e	-13.33±0.00 c	-32.33±0.00 d	-28.12±0.00 b
11	57.14±0.00 bc	63.30±3.33 ab	51.59±1.68 b	46.86±1.31 ab

Notes: Data is expressed as mean ± SE of rate of oviposition-repelling (%) of three replication. Data with the same letter represents the values that are not different statistically (*P* = 0.05) (DMRT). Those in negative values represent attraction effect.

3.3 Dose-response to compounds 6 and 9

The results of EAG investigation showed also that EAG responses of *O. furnacalis* to compounds 6 and 9 were dose-dependent. When treated concentration of compound 6 was increased from 4 to 10 mg/mL, the

values of S_r of the female and the male were from 32.28% to 68.22% and from 30.06% to 105.62%; and when treated concentration of compound 9 was increased from 0.0625 to 1 mg/mL, the values of S_r of the female and the male were from 33.32% to 113.62% and 32.50% to 174.35% respectively (Figs. 2 and 3).

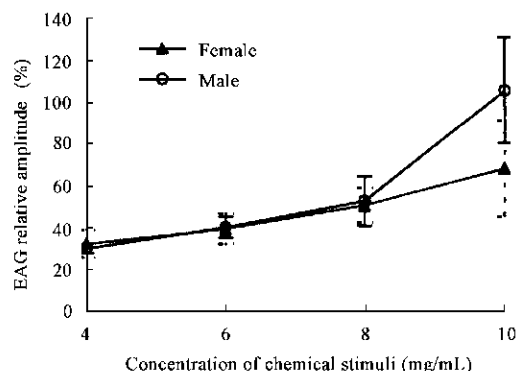


Fig. 2 EAG response curves of *Ostrinia furnacalis* to compound 6 ($n = 6 - 8$)

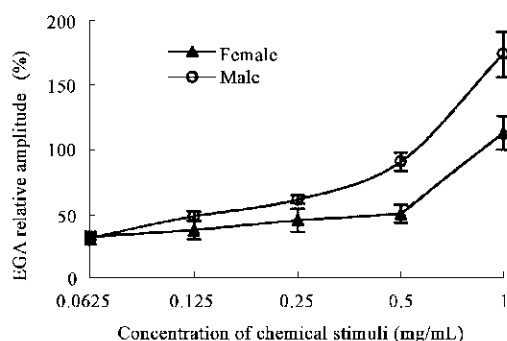


Fig. 3 EAG response curves of *Ostrinia furnacalis* to compound 9 ($n = 6 - 8$)

4 DISCUSSION

4.1 Behavioral response to polyacetylenes

The behavioral assays clearly demonstrated the potentiality of polyacetylene compounds to repel oviposition of *O. furnacalis*. In the 11 compounds tested, compound 9 was one of the most excellent repellents to the adult of *O. furnacalis*, which occurs as a secondary metabolite in the plant of *Artemisia scoparia* naturally, and would play an important role in IPM of the Asian corn borer.

4.2 EAGs to polyacetylenes

It is well known that insect behavioral responses are involved in perception of volatile chemicals which have the effects of luring or repelling to oviposition of insects. Sensory cells to detect volatiles are olfactory receptor neurons located on the antenna of insects. The electroantennogram can record the cumulative antenna

olfactory sensilla responses to volatiles and provide us with a lot of information to understand the behavioral responses of insects. The data of EAGs recorded for the tested polyacetylenes in our experiment demonstrated that these compounds could excite the sensory cells of olfactory sensilla, and indicated also that the sensory cells on antenna might be one of action targets of polyacetylene compounds, at least to compound 9 it is so. According to the results of our experiment, we can infer the excited sensory cells elicited by tested compounds may transduce the messenger to central nervous system, and in turn regulate the behavior of oviposition of *O. furnacalis*.

4.3 The correlation between oviposition-repelling activity and EAGs

In order to identify further the action target of polyacetylenes in repelling activity to the adults of *O. furnacalis*, a correlation was made between electrophysiological investigation and behavioral bioassay by regression analysis of data of two sets. The formula of regression as follow: $y = -38.1989 + 2.2130x$, in which: $r = 0.599$; y : activity of oviposition-repelling (%); x : relative EAGs (S_r).

It was found from " r " that the values of amplitude of depolarization of receptors of antenna induced by tested chemicals were not correlated well with repelling activity in behavioral test. However, we think that despite the EAG can provide a lot of information to understand the mechanism of behavioral response to some compounds, it does not always agree well with behavioral responses of the insect. Some volatiles can elicit EAGs of some insects, but in behavioral test these insects show no behavioral response to them. For example, β -caryophyllene can elicit relatively high EAGs in most insects, but elicits only weak behavioral response of these insects. We infer that there are other action targets besides the receptor of antenna, which may include the chemosensilla on prothoracic tarsi and ovipositor. The effects of polyacetylenes on oviposition through these chemosensilla will be investigated, and moreover the mechanism on sensitivity difference of two sexes, *i. e.*, the male more sensitive than female to compound 6 and 9 on EAGs, will be also studied.

4.4 Relationship of chemical structure and repelling activity

Comparing the bioassay data of oviposition-repelling activity of 11 tested compounds on *O. furnacalis* (Table 2), we can find that the six phenyl-substitute derivatives of four buta-, one penta- and one hexadiyne (Table 1: compounds 4, 5, 6, 7, 8 and 9) had higher activity than the three alky-substitute derivatives (Table 1: compounds 1, 2 and 3). Mono-phenyl substitute hexadiyne (compound 9) had the highest activity than other diphenyl-substitute diacetylene compounds in six phenyl-substitute

derivatives.

In conclusion, the results of this preliminary study show that mono-phenyl substitute hexadiyne compound can be used as a leading-compound for synthesizing new repellent to the pest.

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多炔类化合物对亚洲玉米螟产卵驱避作用 及玉米螟的触角电位反应

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摘要: 以茵陈二炔为结构母体, 人工合成了 11 个多炔类化合物。采用蜡纸接卵法, 测定了 11 个化合物对亚洲玉米螟 *Ostrinia furnacalis* 的产卵驱避活性。结果表明: 当处理浓度为 20 $\mu\text{g}/\text{cm}^2$ 时, 受试的化合物对亚洲玉米螟的产卵行为具有一定程度的驱避作用, 其中化合物 9(1-苯基-4-甲基-丁二炔)对亚洲玉米螟产卵驱避作用明显, 调查处理后的 3 天、4 天、5 天和 6 天, 其产卵驱避率分别为 85.71%、80.00%、61.27% 和 62.51%。触角电位测定表明, 受试的 11 个化合物对亚洲玉米螟成虫触角感受器具有刺激作用, 其中化合物 6 和化合物 9 能强烈地刺激产生高振幅的动作电位。10 mg/mL 浓度处理, 测得化合物 6 触角电位相对值, 雌、雄虫分别为 68.22% 和 106.60%, 化合物 9 分别为 199.19% 和 220.60%。经回归分析所测 11 个化合物的产卵驱避活性与触角电位反应相对值, 两者呈现一定程度上的相关性。还讨论了合成的多炔类化合物对亚洲玉米螟可能的作用靶标和结构与活性间的关系。

关键词: 多炔类化合物; 亚洲玉米螟; 产卵驱避活性; 触角电位

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